CLAIMS

1. A polymer affinity matrix for binding one or more substances in a fluid for removing said substance(s) from the fluid and/or decreasing the amount or concentration thereof in said fluid with a view to preventing, eliminating, or reducing undesired activation of components or processes in said fluid, wherein said matrix comprises

a) a solid support

b) at least one spacer bound to the solid support, and, coupled to each spacer,

c) a ligand containing at least one binding unit having at least one functional group, wherein the polymer affinity matrix has the ability to selectively bind said substance(s).

2. The polymer affinity matrix according to claim 1, wherein said ligand has a defined three-dimensional structure which is complementary as regards charge and/or hydrophobicity/hydrophilicity to the three-dimensional structure of a binding motif of said substance(s),

3. The polymer affinity matrix according to any one of claims 1 and 2, wherein the ligand is represented with the formula

$-X^1{}_n-Y_m[X^2{}_i-Z^1;\ X^3{}_j-Z^2]_{\frac{1}{2}(m+1)}$, (general Formula I), wherein

$n = 0$ or 1;
$m = 2^k - 1$;
$k = 0$ to 10, wherein if $k = 0$ then $X_2 = X_3$ and $Z_1 = Z_2$;
$i = 0$ or 1; and
$j = 0$ or 1,

or

$-(X^1{}_n-Y^1[Y^2{}_m[X^2{}_i-Z^1;\ X^3{}_j-Z^2]_{\frac{1}{2}(m+1)})_r-X^4{}_p-Z^3$, (general Formula II), wherein

n = 0 or 1;
m = 2^k-1;
k = 0-10, wherein if k = 0 then $X_2 = X_3$ and $Z_1 = Z_2$;
r = 1-100;
i = 0 or 1;
j = 0 or 1; and
p = 0 or 1;

wherein Z^1, Z^2 and Z^3 each represents the binding unit and is each an organic molecule chosen from the group consisting of an amino acid, a peptide, a fatty acid, a carbohydrate, a lectin, and a nucleotide, and derivatives thereof, or combinations thereof, wherein Y, Y^1 and Y^2 each is a trifunctional branching molecule chosen from the group consisting of amino, hydroxy, aldehyde, isocyanate, isotiocyanate, thiol, maleimido, epoxy, and derivatives thereof, or combinations thereof, and wherein X^1, X^2, and X^3 each is an optional bifunctional distance molecule containing two functional groups chosen from the group consisting of amino, carboxy, hydroxy, aldehyde, isocyanate, isothiocyanate, thiol, maleimido, epoxy, and derivatives thereof, or combinations thereof;
and/or wherein the ligand is cyclic.

4. The polymer affinity matrix according to any one of the preceding claims, wherein the ligand comprises 1-100 functional groups, preferably 1-32 functional groups.

5. The polymer affinity matrix according to any one of the preceding claims, wherein each binding unit is an amino acid, at least a part of which is positively charged at about physiological pH of blood.

6. The polymer affinity matrix according to claim 5, wherein the amino acid has a pK_A of ≥ 6.0.

7. The polymer affinity matrix according to claim 6, wherein the amino acid is arginine, lysine, histidine, or cysteine, preferably arginine.

8. The polymer affinity matrix according to claim 7, wherein the amount or concentration of the amino acid is 0.01-5 mmol/g matrix.

9. The polymer affinity matrix according to claim 8, wherein the amount or concentration of the amino acid is about 0.01, 0.1, 1, 2, 3, 4 or 5 mmol/g matrix.

10. The polymer affinity matrix according to any one of the preceding claims, wherein the number of amino acid molecules per ligand is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16, preferably 4-8.

11. The polymer affinity matrix according to any one of the preceding claims, wherein the amino acid is arginine and the amount or concentration of arginine is ≤ 3 mmol/g matrix.

12. The polymer affinity matrix according to any one of the preceding claims, wherein said at least one functional group is an amino group or substituted amino group, a carboxy group, a hydroxy group, a thiol group, a guanidino group, or combinations thereof, preferably an amino group or guanidino group.

13. The polymer affinity matrix according to any one of the preceding claims, wherein the ligand has a tree- or comb-like structure as shown in the following formulas:

Examples of ligands having the general Formula I, i.e. $-X^1{}_n-Y_m[X^2{}_i-Z^1; X^3{}_j-Z^2]_{½(m+1)}$ *, wherein it here is* $-Lys_m[Arg]$ *(m+1), i.e.*

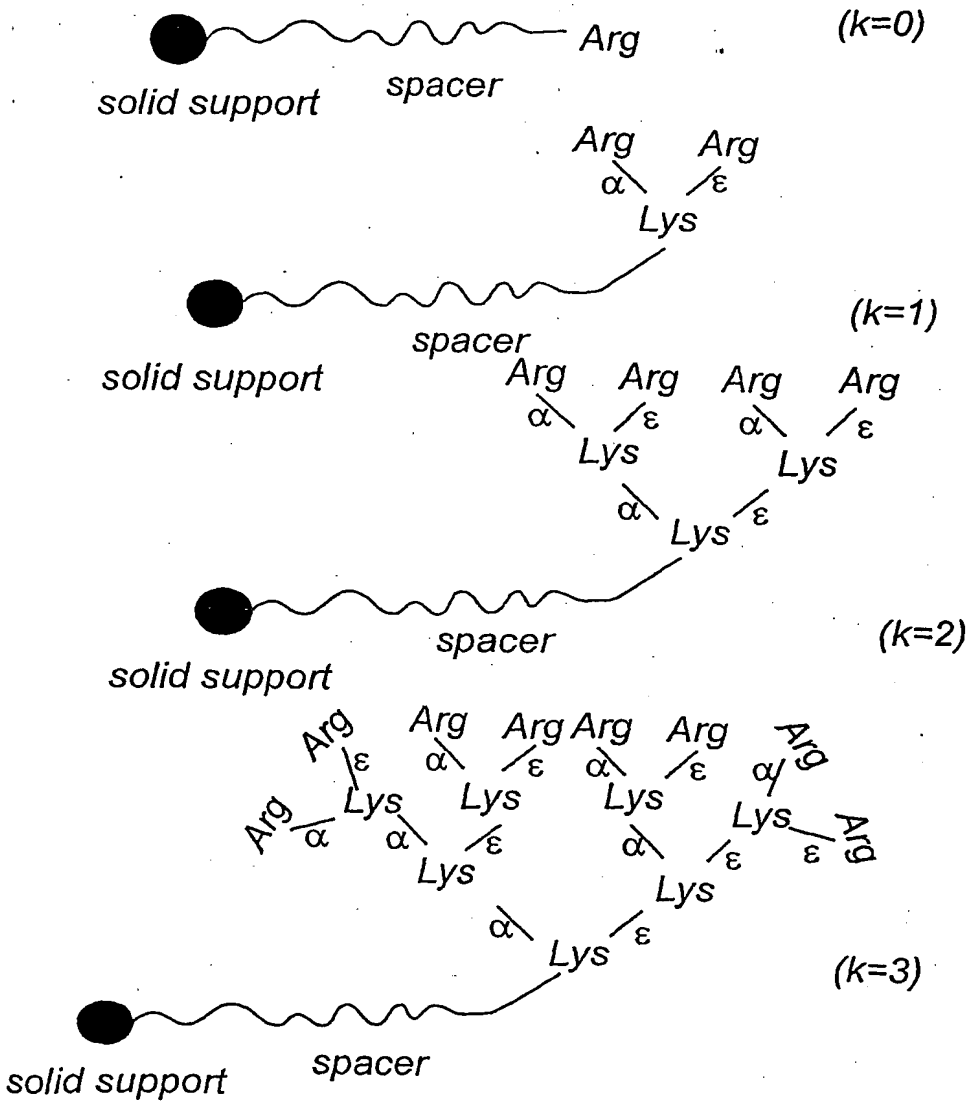
$n = i = j = o;$

$m = 2^k-1$

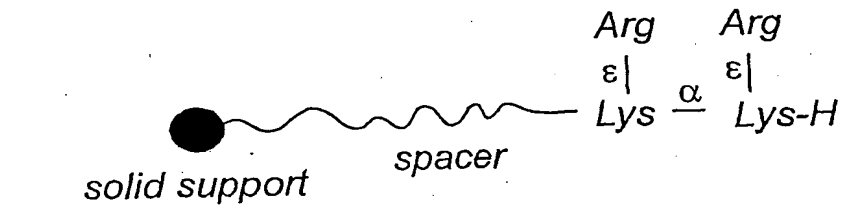
$Z^1 = Z^2 = Arg;$

all Y = Lys; and

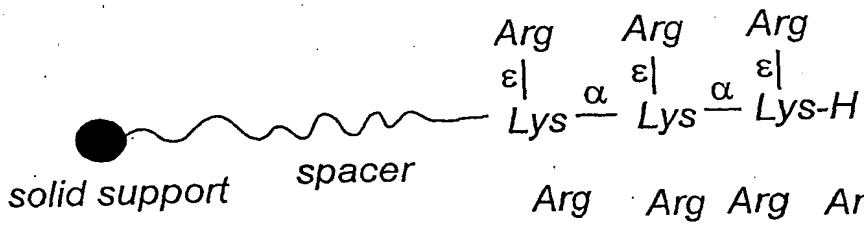
X^1, X^2, *and* X^3 *are absent:*



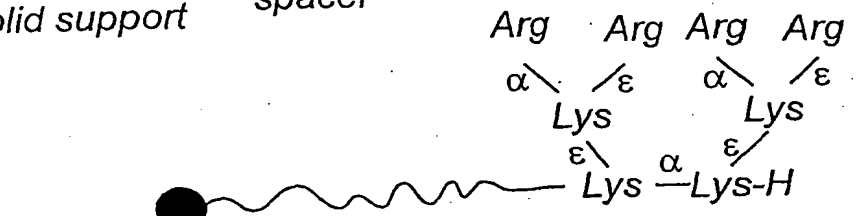
Examples of ligands having the general Formula II, i.e.
$-(X^1_n-Y^1[Y^2_m[X^2_i-Z^1;X^3_j-Z^2]_{½(m+1)})_r-X^4_p-Z^3$, wherein it here is
$-(Lys\,[Lys_m[Arg]_{(m+1)r}-H$, i.e.
$n = i = j = p = 0$;
$m = 2^k-1$
$Z^1 = Z^2 = Arg$;
$Z^3 = H$;
all Y = Lys;
X^1, X^2, X^3 and X^4 are absent:



(r = 2, k = 0)

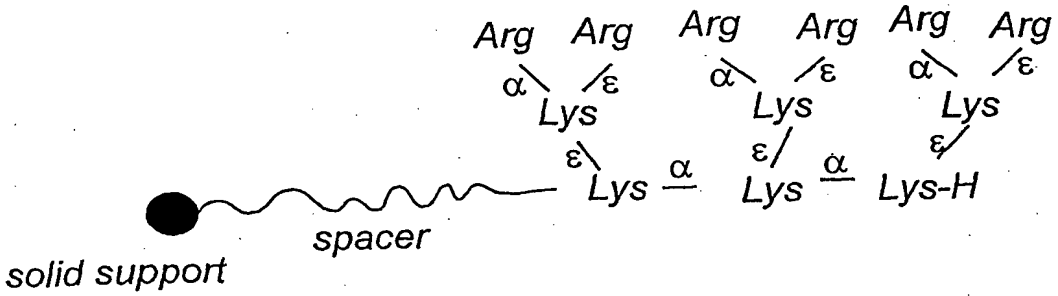


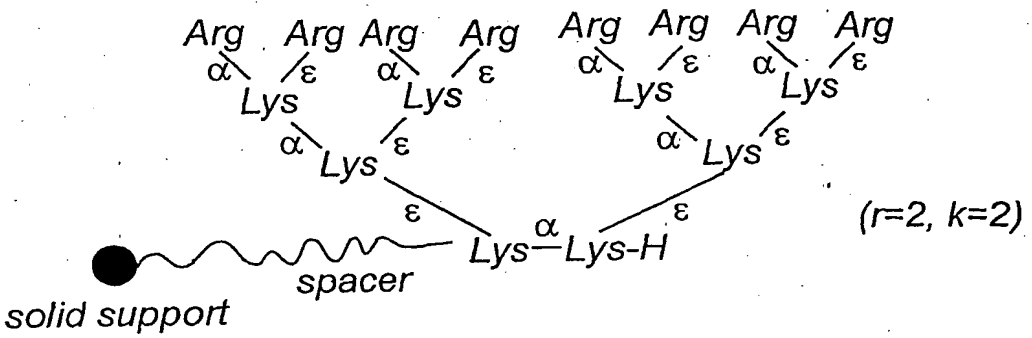
(r = 3, k = 0)

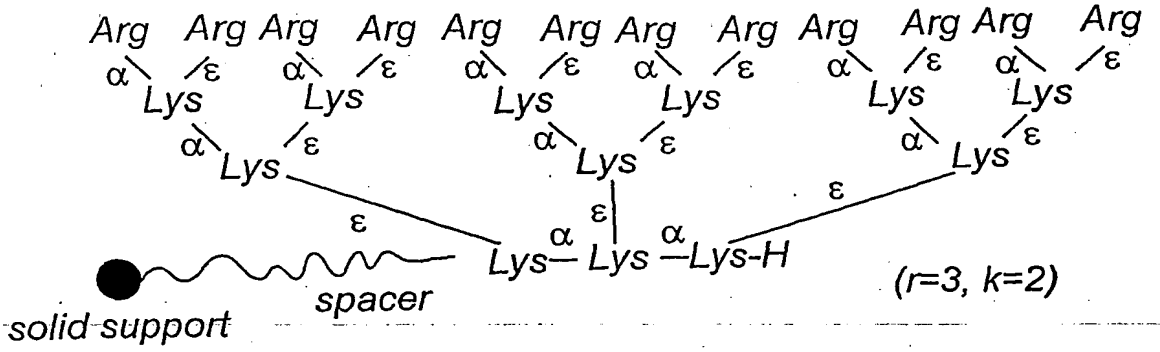


(r = 2, k = 1)

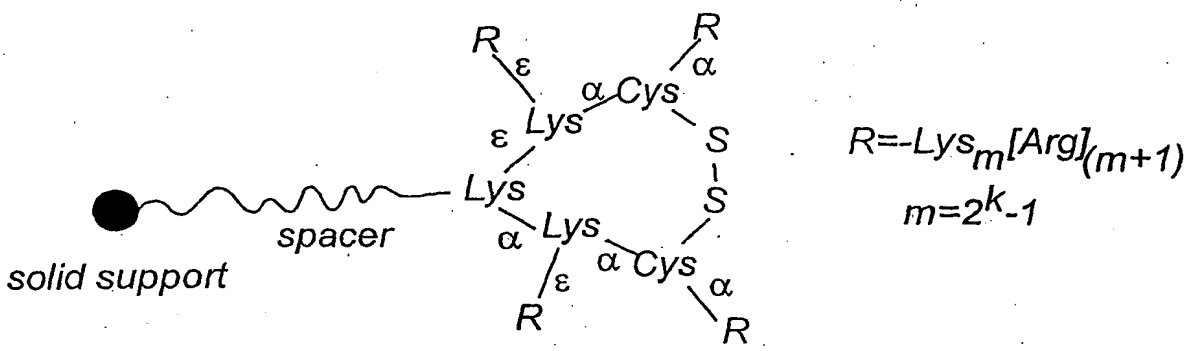
(r = 3, k = 1)

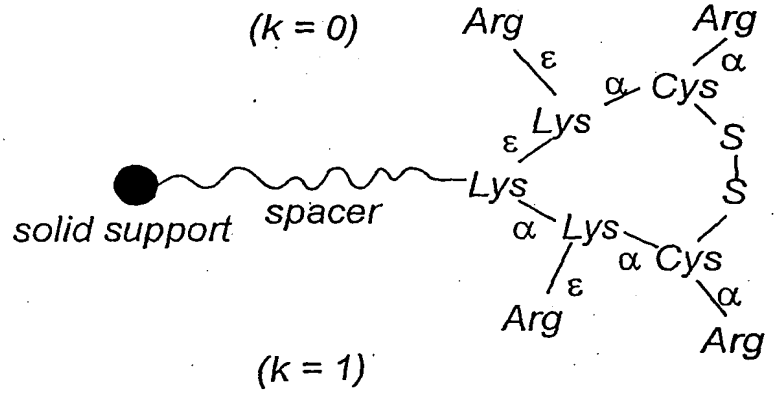


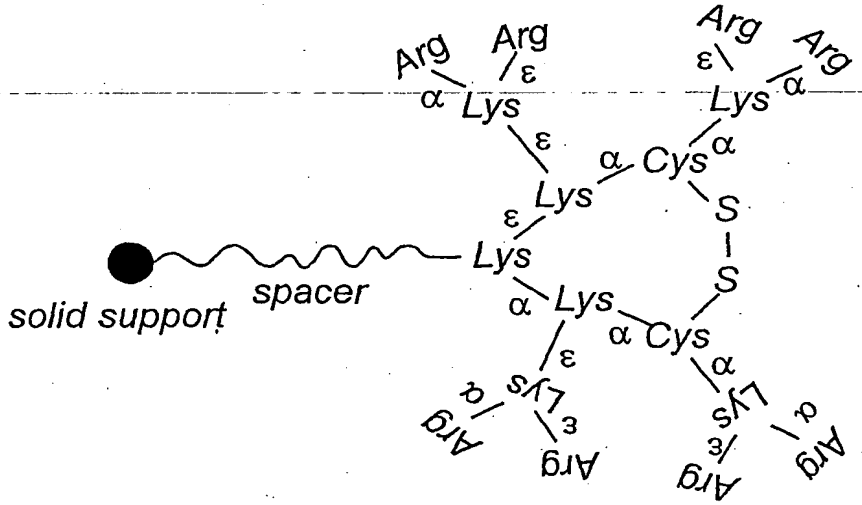

Arg Arg Arg Arg Arg Arg Arg Arg
α ε α ε α ε α ε
Lys Lys Lys Lys
α ε α ε
Lys Lys
ε α ε
Lys Lys-H
spacer
solid support
(r=2, k=2)


Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
α ε α ε α ε α ε α ε α ε
Lys Lys Lys Lys Lys Lys
α ε α ε α ε
Lys Lys Lys
ε ε ε
α α
Lys Lys Lys-H
spacer
solid support
(r=3, k=2)

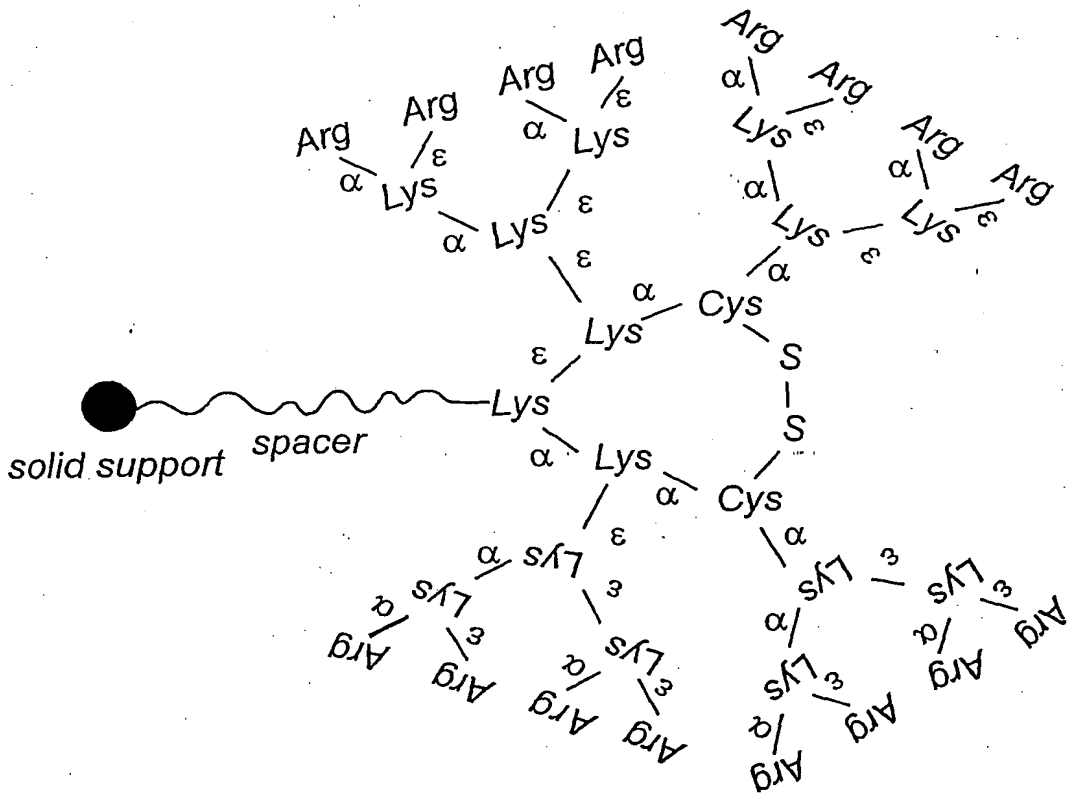
and/or includes a cyclic structure, preferably as shown in the following formulas:







(k=2)



14. The polymer affinity matrix according to any one of the preceding claims, wherein positive charges of at least two of the functional groups are separated from each other by a distance defined by the distance between individually negatively charged groups in the binding motif of the substance(s) to be bound, preferably phosphate groups in an endotoxin.

15. The polymer affinity matrix according to any one of the preceding claims, wherein the spacer is substantially hydrophobic or hydrophilic and has the function of an anchoring part for the ligand.

16. The polymer affinity matrix according to claim 15, wherein the spacer is selected from the group consisting of poly- or oligoethylene glycols of the formula $H-(OCH_2CH_2)_n-OH$, wherein n represents 2-250, or polyvinylalcohols, polyvinylamines, polyolycidoles, polyethyleneimines, polypropyleneoxides, or derivatives thereof.

17. The polymer affinity matrix according to claim 16, wherein the spacer is a polyethylene glycol (PEG) in a linear and/or branched configuration and has an average molecular weight of 400-10 000 Daltons, or derivatives thereof.

18. The polymer affinity matrix according to any one of the preceding claims, wherein the solid support is made of a material selected from the group consisting of polystyrene, polyvinyl alcohols, polyhydroxystyrenes, polymers produced from chloromethylated polystyrenes or polyacrylates, polymethacrylates functionalised with hydroxy groups, hydroxyalkyl-polystyrenes, hydroxyaryl-polystyrenes, hydroxyalkyl-aryl-polystyrenes, polyhydroxyalkylated polystyrenes, polyhydroxyarylated polystyrenes, isocyanatoalkyl-polystyrenes, isocyanatoaryl-polystyrenes, carboxyalkyl-polystyrenes, carboxyaryl-polystyrenes, aminoalkyl-polystyrenes, aminoaryl-polystyrenes, polymethacrylates, cross-linked polyethyleneglycols, cellulose, silica, carbohydrates, latex,

cyclo-olefine copolymers, glass or combinations thereof, preferably a cross-linked polystyrene.

19. The polymer affinity matrix according to claim 18, wherein the solid support has the form of a bead, gel, membrane, particle, net, woven or non-woven fabric, fibre mat, tube, film, foil or combinations thereof or cross-linked interpenetrating networks, preferably a polystyrene-PEG bead.

20. The polymer matrix according to any one of the preceding claims, wherein said matrix is biocompatible and has a swelling capacity enough to allow perfusion of whole blood.

21. The polymer matrix according to claim 20, wherein the swelling capacity is about 1.5-20 fold, preferably 2-6 fold, from a dry state to the hydrated form.

22. The polymer affinity matrix according to any one of the preceding claims, wherein said matrix provides a three-dimensional complementary structure for binding at least one substance selected from the group consisting of bacteria or virus derived constituents, endotoxins, exotoxins, bacterial DNA or fragments thereof, oligonucleotides; cells, in particular endothelial cells, stem cells, and tumour cells; blood cells, in particular lymphocytes, thrombocytes, granulocytes, dendritic cells, and monocytes; prions, parasites, fungi, drugs after overdosing, pathogenic food additives, products from acute or chronic metabolic disturbances resulting from diabetes mellitus, liver disease, uraemia, kidney diseases or inflammation, heparin, bacteria and viruses, pathogen-loaded blood cells, or at least parts or degradation products thereof, DNA, phosphate, cytokines, growth factors, hormones, chemokines, uremic toxins, blood clotting proteins, procoagulatory proteins, inflammatory or proinflammatory proteins, macrophage migration inhibitory factor, soluble or cell surface bound proteins, soluble adhesion molecules, and glucose or degradation products thereof, pyrogens, bacterial

exotoxins, products from Gram-positive bacteria, preferably lipoteichonic acid, in particular bacterial pyrogens, preferably endotoxins, in particular the lipid A component of lipopolysaccharides (LPS).

23. The polymer affinity matrix according to any one of the previous claims, wherein said matrix has a cut-off value of from about 1×10^2 to about 1×10^6 Daltons and binds hydrophobic and/or hydrophilic substances.

24. The polymer affinity matrix according to any one of the preceding claims, wherein the fluid which the substance(s) is/are to be removed from or to be reduced in is an aqueous or organic solution, a body fluid, preferably blood, therapeutical fluids, fluids for life science applications, preferably buffer solutions, infusion fluids or dialysis fluids in biological, diagnostic or biotechnological applications, blood products obtained from healthy donors, such as plasma, platelet concentrates, erythrocyte concentrates which are used for transfusions, blood substitutes, preferably oxygen carriers, modified hemoglobin solutions and artificial hemoglobin solutions; fluids for nutrition and fluids for industrial use.

25. The polymer affinity matrix according to any one of the preceding claims, wherein the solid support is a cross-linked polystyrene, the spacer is a polyethylene glycol and each binding unit is arginine.

26. A method for removing one or more substances from a fluid and/or reducing the amount or concentration thereof with a view to preventing, eliminating or reducing undesired activation of components or processes in said fluid, comprising contacting the fluid with the polymer affinity matrix as defined in any one of claims 1-24 for a period of time sufficient to reduce the amount or concentration and/or remove said substance(s), preferably up to 24 hours.

27. The method according to claim 26, wherein the period of time is from 1 s to 2 hours.

28. The method according to any one of claims 26 and 27, wherein the substance is an endotoxin and the fluid is blood, wherein the amount or concentration of endotoxin after being removed or reduced is below the capacity of activating components in blood or prevents activation of components or processes in blood.

29. A method for producing a polymer affinity matrix as defined in any one of claims 1-25, comprising

a) attaching the spacer to the solid support to obtain a first complex, and

b) attaching to said first complex the ligand containing said at least one binding unit with at least one functional group;

or

c) attaching the spacer to the ligand containing said at least one binding unit with at least one functional group to obtain a second complex, and

d) attaching the solid support to said second complex;

or

e) attaching the spacer to the solid support to obtain a first complex, and

f) solid phase synthesis of the ligand on the spacer bound to the solid support, or

g) building up or synthesizing the spacer from monomers directly on the solid support by grafting, and

h) attaching to said first complex the ligand containing said at least one binding unit with at least one functional group,

or

i) building up or synthesizing the spacer from monomers directly on the solid support by grafting, and

k) solid phase synthesis of the ligand on the spacer bound to the solid support,

wherein information about the three-dimensional structure, presence of charges and hydrophobic/hydrophilic regions of the binding motif on the substance(s) to bind is collected from X-ray crystallography, protein sequencing, protein modelling or hydrophobicity and hydrophilicity calculations and the ligand containing the binding unit is made complementary as regards charge and/or hydrophilicity/hydrophobicity to the binding motif of said substance(s).

30. The method according to claim 29 comprising the steps of,

for a) and b), activation of the solid support, coupling of the spacer molecule on the solid support, synthesis of the ligand containing the binding unit, and site specific coupling of the ligand to the spacer molecule, or,

for c) and d), synthesis of the ligand containing the binding unit, coupling of the spacer molecule to the ligand, activation of the solid support, and site specific coupling of the spacer-ligand complex to the solid support, or,

for e) and f), activation of the solid support, coupling of the spacer molecule to the activated solid support, and solid phase synthesis of the ligand on the spacer bound to the support.

31. The method according to claim 29 comprising the steps of,

for a) and b), activation of the spacer, coupling of the activated spacer to the solid support, and coupling the ligand to said activated spacer, or,

for c) and d), synthesis of the ligand, activation of the spacer, site specific coupling of the ligand to the activated spacer molecule and coupling of the spacer-ligand complex to the solid support, or,

for e) and f), activation of the spacer, coupling of the activated spacer to the solid support and solid

synthesis of the ligand on the spacer bound to the solid support.

32. Use of the polymer affinity matrix as defined in any one of claims 1-25 for removal of one or more substances, preferably endotoxins, from a fluid, or decreasing the amount or concentration thereof in said fluid, preferably a body fluid or a therapeutic fluid, most preferably blood or serum.

33. Use according to claim 32 for production of less activated blood or prevention of undesired activation of components or processes in blood.

34. Use according to claim 33 as a part of an extracorporeal blood purification process or as an implant in the body to contact blood or any body fluid, preferably in the vascular system, blood vessels or the peritoneal cavity.

35. Use according to claim 34 for production of less activated blood or prevention of undesired activation of components or processes in blood.

36. A kit for removing one or more substances from a fluid and/or decreasing the amount or concentration thereof in said fluid with a view to preventing, eliminating, or reducing undesired activation of components or processes in said fluid, said kit comprising a polymer affinity matrix as defined in any one of claims 1-25.

37. Kit according to claim 36, wherein it further comprises sample tubes, and a device for extra- and/or intracorporeal treatment of said fluid, preferably blood or serum.

38. Use of a polymer matrix for the production of a polymer affinity matrix for removal of one or more substances from a fluid or decreasing the amount or concentration thereof in said fluid,

wherein the specific affinity is dependent on any ligand applied on the polymer matrix,

wherein the polymer matrix includes a solid support and a spacer,

wherein the solid support is made of a material selected from the group consisting of polystyrene, polyvinyl alcohols, polyhydroxystyrenes, polymers produced from chloromethylated polystyrenes or polyacrylates, polymethacrylates functionalised with hydroxy groups, hydroxyalkyl-polystyrenes, hydroxyaryl-polystyrenes, hydroxyalkyl-aryl-polystyrenes, polyhydroxyalkylated polystyrenes, polyhydroxyarylated polystyrenes, isocyanatoalkyl-polystyrenes, isocyanatoaryl-polystyrenes, carboxyalkyl-polystyrenes, carboxyaryl-polystyrenes, aminoalkyl-polystyrenes, aminoaryl-polystyrenes, polymethacrylates, cross-linked polyethyleneglycols, cellulose, silica, carbohydrates, latex, cyclo-olefine copolymers, glass or combinations thereof, preferably a cross-linked polystyrene, and

wherein the spacer is selected from the group consisting of poly- or oligoethylene glycols of the formula $H-(OCH_2CH_2)_n-OH$, wherein n represents 2-250.

39. Use according to claim 38, wherein the solid support has the form of a bead, gel, membrane, particle, net, woven or non-woven fabric, fibre mat, tube, film, foil or combinations thereof or cross-linked interpenetrating networks.

40. Use according to claim 38 or 39, wherein the spacer is a polyethylene glycol (PEG) in a linear and/or branched configuration and has an average molecular weight of 400-10 000 Daltons, or derivatives thereof.

41. Use according to any one of claims 38-40, wherein the polymer matrix has a swelling capacity enough to allow perfusion of plasma or whole blood.

42. Use according to claim 41, wherein the swelling capacity is about 1.5-20 fold, preferably 2-6 fold, from a dry state to the hydrated form.

43. Use according to any one of claims 38-42, wherein the polymer matrix has the form of gel type beads.

44. Use according to any one of claims 38-43, wherein said fluid is an aqueous or organic solution, a body

fluid, preferably blood, therapeutic fluids, fluids for life science applications, preferably buffer solutions, infusion fluids or dialysis fluids in biological, diagnostic or biotechnological application, blood products obtained from healthy donors, such as plasma, platelet concentrates, erythrocyte concentrates, preferably oxygen carriers, modified hemoglobin solutions and artificial hemoglobulin solutions, fluids for nutrition and fluids for industrial use.

45. Use according to any one of claims 38-44, wherein said polymer matrix has a cut-off value of from about 1 x 10^2 to about 1 x 10^6 Daltons and binds hydrophobic and/or hydrophilic substances.

46. Use according to any one of claims 38-45, wherein the solid support is a cross-linked polystyrene, and the spacer is a polyethylene glycol.